PATENT SPECIFICATION (11) 1 414 479

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(21) Application No. 58661/72 (22) Filed 19 Dec. 1972 (19)
(31) Convention Application No. 210 511 (32) Filed 21 Dec. 1971 in
(33) United States of America (US)
(44) Complete Specification published 19 Nov. 1975
(51) INT. CL.² G01N 33/16 23/00
(52) Index at acceptance
G1B 11E 17F 19X 22A

(54) TEST APPARATUS FOR DIRECT RADIOIMMUNOASSAY FOR ANTIGENS AND THEIR ANTIBODIES

(71) We, ABBOTT LABORATORIES, a Corporation organised and existing under the laws of the State of Illinois, United States of America, of 14th Street and Sheridan Road, North Chicago, County of Lake, State of Illinois, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to test apparatus for use in radioimmunoassay for antigens or their antibodies. More particularly, this invention relates to test apparatus for a direct radioimmunoassay for determining hepatitis associated antigen or its antibody or antigens and antibodies having at least two free antigenically active sites.

Although there have been methods for determining the presence of antigenically active macromolecules such as intact viruses, virus capsids, virus subunits, bacteria, membranes, cell walls, hormones, etc., there has been a lack of a simple, yet sensitive, test method and apparatus for determining the presence of these materials. Viral hepatitis, including so-called serum hepatitis, which is a relatively common disease, has not been heretofore easily detected by a sensitive test which is both specific and reproducible for quickly determining whether or not the serum from a patient or a donor contains hepatitis associated antigens or antibodies.

Furthermore, radioimmunoassay techniques have been developed in the past for various antigen-antibody materials; however, these radioimmunoassay techniques such as disclosed in articles by Kevin Catt et al in the Journal of Biochemistry, 1966, Volume 100, page 31c and 33c and in Science, Volume 158, page 1570, 1967, are an indirect radioimmunoassay technique wherein the amount of antigen present is roughly inversely proportional to the amount of radiation emitted by the tracer material. These procedures require the use of correlation tables and other materials which generally rendered the results less than reproducible and exact.

Briefly, it has been discovered that the above-noted difficulties, i.e. lack of reproducibility and exactness, can be substantially reduced or overcome by utilizing the diagnostic apparatus of the present invention with the associated radioimmunoassay technique.

According to the present invention test apparatus for use in the direct radioimmunoassay for antigens or their antibodies comprises:

(a) a receptacle for receiving a sample to be assayed, and

(b) a test reagent-carrying member comprising a handle portion and an end portion, which end portion is insertable within the receptacle and has a coating thereon of an antigen or of the corresponding antibody which, in use of the apparatus, binds the corresponding antibody or antigen present in said test sample.

In order that the invention may be more readily understood, reference will now be made to the accompanying drawings, in which:—

Figure 1 is an exploded perspective view of one embodiment of test apparatus according to the present invention;

Figure 2 is a part-sectional view of the apparatus shown in Figure 1 after assembly;

Figure 3 is an exploded perspective view of a second embodiment of the test apparatus of the present invention;

Figure 4 is a part-sectional view of the embodiment shown in Figure 3 after assembly;

Figure 5 is an exploded perspective view of a third embodiment of the present invention;

Figure 6 is a part-sectional view of the assembled apparatus shown in Figure 5; and

Figure 7 is a perspective view showing an alternative configuration for the handle portion of the test apparatus.

Referring to Figures 1 and 2 the test apparatus comprises a test reagent-carrying

member 1 and two testing receptacles 3 and 5. Member 1 comprises a handle portion in the form of a solid rod portion 15 having an end portion in the form of a frusto-conical shaped tip 2. The distal end 12 of rod portion 15 is usually flat although its shape is not particularly important. Rod portion 15 is generally cylindrical in shape and may be either solid as shown in Figure 1 or it may be hollow. Rod portion 15 and tip 2 may be made from any material which is capable of being shaped, such as glass, polystyrene or polypropylene. Since the test apparatus is generally designed for a single test only and is disposable, various plastics materials such as polystyrene and polypropylene are preferred materials of construction. As shown in Figures 1 and 2 the test reagent-carrying member 1 comprises a rod portion 15 which is integral with the tip 2. However, the rod portion 15 and the tip 2 may be made separately and joined by any suitable method such as adhesive bonding. The tip 2 is coated with hepatitis associated antigen or with its antibody. The method of coating the tip 2 will be discussed in detail below. Associated with member 1 is at least one test receptacle 3, although since the test procedure requires two separate test receptacles, the apparatus is usually provided with two test receptacles 3 and 5 fitted to opposite ends of rod portion 15 as shown in Figure 2. Test receptacle 3 comprises an outer wall 6 which is generally cylindrical in configuration, a base 13, an inner cylindrical wall 8, a frusto-conical well portion 4 and an annular lip 7. The internal diameter of cylindrical wall 8, which is approximately one-half the length of outer wall 6 is slightly greater than the diameter of rod portion 15. Annular lip 7 defines the upper boundary of the conical well portion 4 and mates with lip 11 of member 1. Well portion 4 can be any shape which ensures intimate contact of the test fluid and the coated tip 2, such as conical, frusto-conical or hemispherical. Of course, coated tip 2 should have a similar configuration and should be slightly smaller than well portion 4 so that the fluid is forced into the gap left between well portion 4 and tip 2 when inserted. It is especially important that well portion 4 is slightly larger than coated tip 2 since this ensures complete contact of the serum to be tested with the coating upon coated tip 2.

Test receptacle 5 is initially mounted on the distal end 12 of rod portion 15 with its annular lip 9 and cylindrical inner wall 10 fitting snugly over the outer surface of rod portion 15 and distal end 12 of member 1. This snug fit is necessary to ensure that well portion 14 and test receptacle 5 are maintained in as sterile a condition as possible. Test receptacle 5 also has a base 16.

Although the diagnostic apparatus of the present invention can be used for determining the presence of any appropriate antigen or its antibody by a simple positive-negative technique, the use of the apparatus will now be described with respect to a procedure for determining the presence of hepatitis associated antigen.

Test reagent-carrying member 1 is initially removed from test receptacle 3 and a sample of blood or serum is placed in well portion 4. If the test is to be conducted for hepatitis associated antigen, a member 1 with a coated tip 2 is utilized wherein the coating comprises antibody to hepatitis associated antigen. Member 1 is replaced in test receptacle 3 so that the coated tip 2 is in intimate contact with the serum contained in well portion 4. The apparatus is then set aside and incubated for a predetermined period of time. Following this incubation, test receptacle 5 is removed from member 1 and radioactively labelled or tagged antibody to hepatitis associated antigen is placed therein. Member 1 is then removed from test receptacle 3, washed and rinsed and placed in test receptacle 5 so that the coated tip 2 is in intimate contact with the tagged antibody to hepatitis associated antigen. The member 1 and test receptacle 5 are then incubated for an additional period of time. Test receptacle 3 should be covered during this incubation process so that the serum which remains in well portion 4 is not contaminated. Following the incubation period, member 1 is removed from test receptacle 5 and coated tip 2 is rinsed. Member 1 is then placed in test receptacle 3 and member 1 and test receptacle 3 are placed in the well of any conventional apparatus designed to count gamma radiation. The amount of radiation is recorded and compared with a blank sample which has been run simultanously, in order to determine the background count rate. A test sample with a count rate above that of the background rate would be considered antigen positive thus providing a simple positive-negative test without resorting to correlation tables and graphs.

Figures 3 and 4 show a second embodiment of the apparatus which comprises a test reagent-carrying member 21 comprising at least two separate parts, a handle portion 31 and an end portion in the form of a coated tip 22. Handle portion 31 may be either integrally molded to form a handle portion with a cross-section in the shape of an X or, as shown in Figure 3, handle portion 31 may comprise two rectangular pieces 23 and 30, each having a small rectangular slit 32 and 33 respectively therein so that

handle portion parts 23 and 30 may be slid together to form the X-shaped handle portion 31. Handle portion 31 may be attached to coated tip 22 by any conventional joining method such as adhesive bonding or alternatively the entire assembly may be molded by any suitable method. Again, member 21 may be made from any material which can be formed into the desired shape with polystyrene and polypropylene being preferred. Coated tip 22 is shown as having a pointed conical configuration as opposed to the frusto-conical tip 2 as shown in Figures 1 and 2. Any similar configuration can be utilized as long as the well with which this tip is to be utilized has a similar configuration.

Test receptacle 24 is an alternative form of the test receptacles as shown in Figures 1 and 2. Test receptacle 24 has an outer cylindrical wall 35 and a pair of inner cylindrical walls 26 and 28. Test receptacle 24 has a well portion 25 and an annular lip 27 having a similar configuration to well portion 4 and annular lip 7 as shown in Figures 1 and 2. Whereas the test receptacles as shown in Figures 1 and 2 have a solid base beneath the well, in test receptacle 24 this portion is hollow, the space being defined by inner wall 28 and the outer wall 29 of well 25. This type of test receptacle utilizes less materials and is preferred since the apparatus is designed for a single use. Test receptacle 24 rests on annular edge 34 which forms the bottom end of the outer cylinder wall 35. Although not shown in Figures 3 and 4, test receptacle 24 may be designed in such a way that these test receptacles may be nested, i.e. placed one within the other. This may be done by utilizing a test receptacle with a stepped or slanted sidewall such that the upper portion of the outer wall 35 will match inner wall 28. Again, as with coated tip 2 and well portion 4 in Figures 1 and 2, well portion 25 should be slightly larger than coated tip 22 so that when the coated tip 22 is placed in well portion 25, the sample contained therein is displaced up the sides of the tip and the well portion so as to ensure intimate contact between the coating on coated tip 22 and the material to be tested in well portion 25.

Figures 5 and 6 show yet another embodiment of the apparatus according to the present invention. This embodiment generally comprises a test receptacle 41 which has a cylindrical inner wall 44 and outer wall 43 and a flat bottom 42. The member 49 of the test apparatus comprises a handle portion 40 and an X cross-sectionally shaped coated end portion 46. The handle portion 40 and the coated end portion 46 may either be integrally formed or separately formed and joined by any suitable conventional method. Coated end portion 46 has a width substantially similar to but slightly less than the diameter of the inner wall 44 of the test receptacle 41. The bottom of coated end portion 46 presents four angularly shaped cuts 45 so that the serum which is placed in test receptacle 41 can freely contact all of coated end portion 46. In utilizing the apparatus shown in Figures 5 and 6, the test sample is placed in the bottom of test receptacle 41 and the handle portion 40 is placed therein, followed by agitation. The apparatus as shown in Figures 5 and 6 is particularly well designed for use where larger quantities of serum are available. When smaller quantities of serum are available, however, the apparatus shown in Figures 1, 2, 3 and 4 is preferred since this apparatus ensures intimate contact between the coated test portion and the unknown sample.

Figure 7 shows a member having an alternative handle portion 51 which may be utilized in place of handle portion 31. Handle 51 is simply a rectangular sheet of material, such as polystyrene or polypropylene, and is directly attached to a hemispherical tip 52 to form the member. Handle 51 may be integrally formed with coated tip 52 or may be joined thereto by any conventional means such as adhesive bonding. The hemispherical tip 52 of the member fits into a cylindrical receptacle having a corresponding hemispherical wall-portion (not shown).

A method of coating end portions 2, 22, 52 and 46 with either an antigen or its antibody will now be described, by way of example, using a hepatitis associated antibody solution of antibody to australia antigen having a concentration of from about 1 to about 100 micrograms of protein per ml. which is prepared in from about 0.005 to about 0.02 milar Tris-HCl, i.e. 2-amino-2-hydroxymethyl-1,3-propanediol-HCl, utilizing an antibody to australia antigen serum. The Tris-HCl is used to buffer the antibody solution to a pH of from about 7.1 to about 9.5 and contains from about 0.01% to about 0.05% sodium azide. One ml. of this hepatitis associated antibody is then coated on the end portions by incubating at room temperature for from 6 to 72 hours. These coated end portions are then washed with a washing medium containing about 0.005 to about 0.02 molar Tris-HCl having a pH of 6.9 to 8.4 and containing from about 0.01% to about 0.05% of sodium azide. Following this washing and rinsing step, the test reagent-carrying member may be stored at 4°C. until necessary for use for radioimmunoassay.

It is preferred to utilize a 0.01 molar solution of Tris-HCl and 0.02% sodium azide buffered at a pH of 7.1 for both the antibody to australia antigen buffered medium and the washing medium.

The amount of antibody coated on the

members is not critical since the test is run each time in comparison with a blank test. No standard curves or charts are necessary for carrying out the test, therefore, no specific amount of antibody in the coating is required as long as two similarly coated members are used.

Furthermore, since the direct radioimmunoassay is often conducted simultaneously with blank tests for comparative purposes, the members shown in Figures 1, 2, 3, 4, 5, 6 and 7 may be molded in sheets connected at the handle end. These combined members may be separated prior or subsequent to coating with the antigen or its antibody in order to facilitate coating and inventory or they may be separated into groups of 2, 3 or 4 members or whatever number is convenient for use with a series of joined test receptacles. These test receptacles may be a sheet with a series of spaced well portions or may be a series of the wells as shown in Figures 1 to 6 which have not been separated subsequent to molding. The use of multiple members has the advantage that the incubation times for the unknown samples will be identical, thus ensuring an accurate comparison.

In our copending Application 58662/72 (Serial No. 1,414,480) we claim a method for determining the presence of antigens or their antibodies in an unknown sample utilizing direct radioimmunoassay comprising:

(a) contacting said unknown sample with a coating of an antigen or its antibody on a solid substrate;

(b) incubating said unknown sample a first time while in contact with said coating;

(c) washing said incubated coating;

(d) contacting said washed coating with a radioactive tracer labelled purified material, said material being either said antigen or its antibody, provided that for determination of antibody said coating is the corresponding antigen and said radioactive tracer labelled material is also said antigen, and vice versa for determination of antigen;

(e) incubating said washed coating a second time while in contact with said radioactive tracer labelled material;

(f) washing the radioactive tracer labelled incubated coating so formed;

(g) counting radiation emitted by said radioactive tracer labelling coating; and

(h) comparing the number of counts from said coating with the number of counts from a control sample which is known to be free from antigen or antibody and which has been subjected to steps (a) to (g).

WHAT WE CLAIM IS:—

1. Test apparatus for use in the direct radioimmunoassay for antigens or their antibodies comprising:

(a) receptacle for receiving a sample to be assayed, and

(b) a test reagent-carrying member comprising a handle portion and an end portion, which end portion is insertable within the receptacle and has a coating thereon of an antigen or of the corresponding antibody which, in use of the apparatus, binds the corresponding antibody or antigen present in said test sample.

2. Apparatus as claimed in claimed in claim 1, in which the end portion of the test reagent-carrying member is coated with hepatitis associated antigen or its antibody.

3. Apparatus as claimed in either of claims 1 or 2, wherein the receptacle comprises a well portion for receiving the sample to be assayed, the well portion and the end portion having a similar shape and the well portion being slightly larger than the end portion.

4. Apparatus as claimed in claim 3, wherein the surface of the well portion and the surface of the end portion are both conical in shape.

5. Apparatus as claimed in claim 3, wherein the surface of the well portion and the surface of the end portion are both frusto-conical in shape.

6. Apparatus as claimed in claim 3, wherein the surface of the well portion and the surface of the end portion are both hemispherical in shape.

7. Apparatus as claimed in any of the preceding claims, wherein the receptacle and the test reagent-carrying member are made of polystyrene or polypropylene.

8. Test apparatus for use in the direct radioimmunoassay for antigens or their antibodies substantially as hereinbefore described with reference to Figs. 1 & 2, Figs. 3 & 4, Figs. 5 & 6, or Fig. 7 of the accompanying drawings.

BARON & WARREN,
16, Kensington Square,
London, W.8,
Chartered Patent Agents,

Printed for Her Majesty's Stationery Office by Burgess & Son (Abingdon), Ltd.—1975.
Published at The Patent Office, 25 Southampton Buildings, London, WC2A 1AY
from which copies may be obtained.

1414479 COMPLETE SPECIFICATION

2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 1

FIG. 1

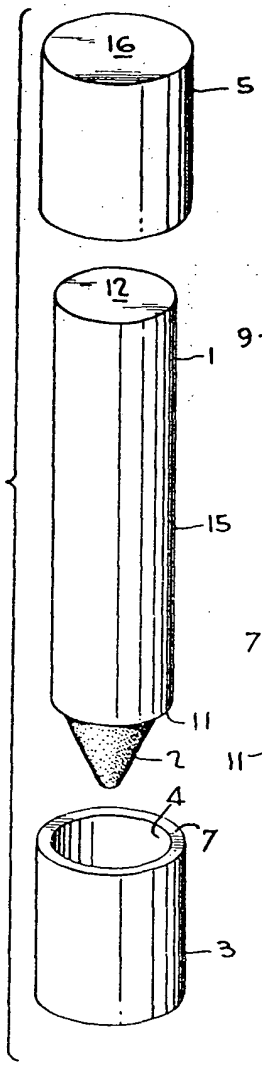


FIG. 2

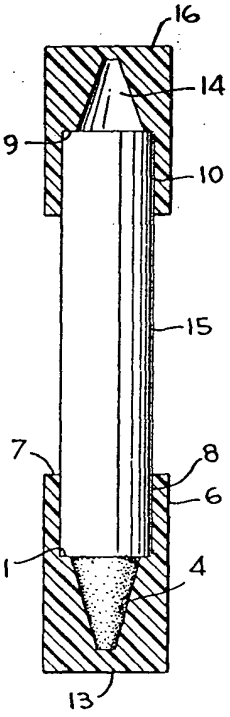


FIG. 3

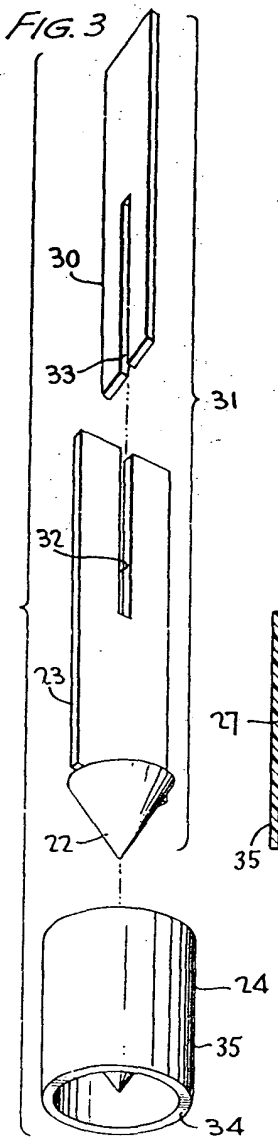
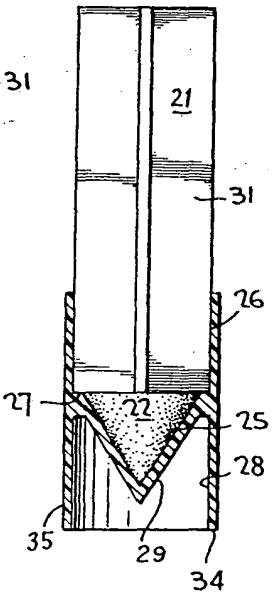


FIG. 4



1414479 COMPLETE SPECIFICATION
2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 2

FIG. 5

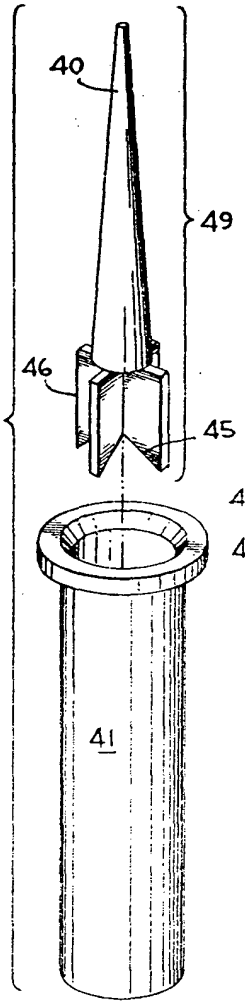


FIG. 6

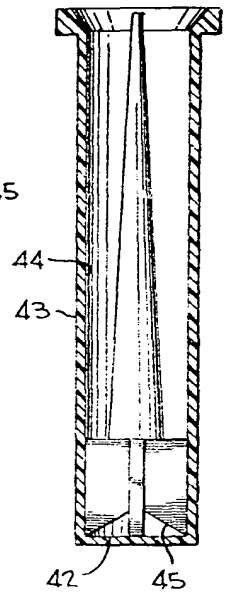


FIG. 7